

What is claimed:

1. A modified integrin I-domain polypeptide containing at least one disulfide bond, such that said modified I-domain polypeptide is stabilized in a desired conformation.
2. A modified integrin I-domain polypeptide of claim 1 which is stabilized in the open conformation.
3. A modified integrin I-domain polypeptide of claim 1 which is stabilized in the closed conformation.
4. A modified integrin I-domain polypeptide of claim 2 which binds ligand with high affinity.
5. A modified integrin I-domain polypeptide of claim 1 which is encoded by an amino acid sequence containing at least one cysteine substitution as compared to the wild-type sequence.
6. A modified integrin I-domain polypeptide of claim 2, wherein the distance between C β carbons of the residues that are substituted for cysteines is 3.00-8.09Å.
7. A modified integrin I-domain polypeptide of claim 1 which is derived from an I-domain of an integrin α subunit selected from the group consisting of: α 1, α 2, α 10, α 11, α D, α E, α L (CD11a), α M (CD11b) and α X (CD11c).
8. A modified integrin I-domain polypeptide of claim 2 which is derived from the I-domain of the α L subunit of LFA-1.
9. A modified integrin I-domain polypeptide of claim 3 which is derived from the I-domain of the α L subunit of LFA-1.

10. A modified integrin I-domain polypeptide of claim 7 which contains amino acid substitutions selected from the group consisting of K287C/K294C, E284C/E301C, L161C/F299C, K160C/F299C, and L161C/T300C. / 2

5 11. A modified integrin I-domain polypeptide of claim 8 which contains amino acid substitutions L289C/K294C.

12. A modified integrin I-domain polypeptide of claim 2 which is derived from the I-domain of the α M subunit of Mac-1.

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13. A modified integrin I-domain polypeptide of claim 3 which is derived from the I-domain of the α M subunit of Mac-1.

14. A modified integrin I-domain polypeptide of claim 12 which contains amino acid substitutions selected from the group consisting of Q163C/Q309C and D294C/Q311C.

15 15. A modified integrin I-domain polypeptide of claim 13 which contains amino acid substitutions Q163C/R313C.

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16. A modified integrin I-domain polypeptide of claim 1 which is comprised within an integrin α subunit.

17. A modified integrin I-domain polypeptide of claim 16 which is further associated with an integrin β subunit.

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18. A modified integrin I-domain polypeptide of claim 1 which is a soluble polypeptide.

19. A modified integrin I-domain polypeptide of claim 1 which is operatively linked to a heterologous polypeptide.

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20. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a modified integrin I-domain polypeptide as defined in any one of claims 1-15.

21. A composition comprising a modified integrin I-domain polypeptide as
5 defined in any one of claims 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 and a pharmaceutically acceptable carrier.

22. A composition of claim 20, wherein said modified integrin I-domain polypeptide is a soluble polypeptide.

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23. A composition of claim 21, further comprising an anti-inflammatory or immunosuppressive agent.

24. The use of a modified integrin I-domain polypeptide of claim 2 as an
15 immunogen to produce antibodies that selectively bind to an integrin I-domain in the open conformation.

25. An antibody, or an antigen binding fragment thereof, which selectively binds to a modified integrin I-domain in the open conformation.

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26. An antibody of claim 25 which binds to an activation specific epitope on the integrin I-domain.

27. An antibody of claim 25 which blocks an interaction between an integrin
25 and a cognate ligand.

28. An antibody of claim 25, or an antigen binding fragment thereof, further comprising a pharmaceutical composition and a pharmaceutically acceptable carrier.

29. An antibody of claim 25, or an antigen binding fragment thereof, wherein
30 said antibody is an LFA-1 antibody.

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30. An anti-LFA-1 antibody, or an antigen binding fragment thereof, which selectively binds to an LFA-1 I-domain in the open conformation.

31. The LFA-1 antibody of claim 30, wherein said anti-LFA-1 antibody, or an
5 antigen binding fragment thereof, selectively binds to a modified LFA-1 I-domain.

32. A modified integrin I-like domain polypeptide containing at least one disulfide bond, such that said modified I-like domain polypeptide is stabilized in a desired conformation.

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33. A modified integrin I-like domain polypeptide of claim 30 which is stabilized in the open conformation.

34. A modified integrin I-like domain polypeptide of claim 31 which binds
15 ligand with high affinity.

35. A modified integrin I-like domain polypeptide of claim 30 which is encoded by an amino acid sequence containing at least one cysteine substitution as compared to the wild-type sequence.

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36. A modified integrin I-like domain polypeptide of claim 30 which is derived from an I-like domain of an integrin β subunit.

37. A modified integrin I-like domain polypeptide of claim 30 which is
25 comprised within an integrin β subunit.

38. A method for stabilizing a polypeptide in a desired conformation, said method comprising introducing at least one disulfide bond into the polypeptide such that the polypeptide is stabilized in a desired conformation.

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39. The method of claim 38, wherein the disulfide bond is formed by the introduction of at least one cysteine substitution into the amino acid sequence of the polypeptide.

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40. The method of claim 38, wherein the distance between C β carbons in the residues that are substituted for cysteines is 3.00-8.09Å.

5 41. The method of claim 38, wherein said polypeptide comprises a functional domain of a protein.

 42. The method of claim 41, wherein said polypeptide comprises an integrin I-domain.

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 43. The method of claim 38, wherein said polypeptide is selected from the group of polypeptides consisting of: an integrin subunit, a small G protein, a heterotrimeric G protein alpha subunit, a tyrosine kinases, a G protein-coupled receptor, an enzyme under allosteric control, a zymogen, complement C3,
15 complement C4, and fibrinogen.

 44. A method for identifying a modulator of integrin activity comprising:
 (a) providing a modified integrin I-domain polypeptide of claim 2;
 (b) contacting the modified integrin I-domain polypeptide with a test compound;

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 (c) assaying the ability of the test compound to bind to the modified integrin I-domain polypeptide,
to thereby identify a modulator of integrin activity.

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45. A method for identifying a compound capable of modulating the interaction of an integrin and a cognate ligand comprising the steps of:

- (a) providing a modified integrin I-domain polypeptide of claim 2;
 - (b) contacting the modified integrin I-domain polypeptide with a ligand of the integrin in the presence and absence of a test compound; and
 - (c) detecting binding between the modified integrin I-domain polypeptide and said ligand,
- to thereby identify a compound capable of modulating the interaction between an integrin and a cognate ligand.

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46. A method for treating or preventing an integrin-mediated disorder in a subject comprising administering to said subject a therapeutically effective amount of a modified integrin I-domain polypeptide stabilized in the open conformation, thereby treating or preventing an integrin-associated disorder in a subject.

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47. The method of claim 46, wherein said integrin-mediated disorder is an inflammatory disorder.

48. The method of claim 46, wherein said integrin-mediated disorder is an autoimmune disorder.

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49. A method of inhibiting the binding of an integrin to a cognate ligand in a subject comprising administering to said subject an effective amount of a modified integrin I-domain polypeptide stabilized in the open conformation, thereby inhibiting the binding of an integrin to a cognate ligand in a subject.

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50. The method of either one of claims 46 and 49, wherein said modified integrin I-domain polypeptide binds ligand with high affinity.

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51. The method of either one of claims 46 and 49, wherein said modified integrin I-domain polypeptide is a soluble polypeptide.

52. The method of claim 50, wherein said modified integrin I-domain polypeptide is operatively linked to a heterologous polypeptide.

53. The method of either of claims 46 and 49, wherein said modified integrin I-domain polypeptide is selected from the group consisting of: α L K287C/K294C, α L E284C/E301C, α L L161C/F299C, α L K160C/F299C, α L L161C/Y300C, α M Q163C/Q309C and α M D294C/Q311C.

54. A method for treating or preventing an integrin-mediated disorder in a subject comprising administering to said subject a therapeutically effective amount of an antibody, or an antigen binding fragment thereof, which selectively binds to an integrin I-domain in the open conformation, thereby treating or preventing an integrin-associated disorder in a subject.

55. The method of claim 54, wherein the antibody binds to a modified integrin I-domain, or an antigen binding fragment thereof.

56. The method of claim 54, wherein said antibody is an LFA-1 antibody, or an antigen binding fragment thereof.

57. The method of claim 54, wherein said integrin-mediated disorder is an inflammatory disorder.

58. The method of claim 54, wherein said integrin-mediated disorder is an autoimmune disorder.

59. A method of treating an integrin-mediated disorder in a subject comprising administering to said subject a therapeutically effective amount of an anti-LFA-1 antibody, or an antigen binding fragment thereof, which selectively binds to an integrin I-domain in the open conformation, thereby treating or preventing an integrin-associated disorder in a subject.

60. The method of claim 59, wherein said anti-LFA-1 antibody binds to a modified LFA-1 I-domain, or an antigen binding fragment thereof.

61. The method of claim 59, wherein said integrin-mediated disorder is an inflammatory disorder.

62. A method of inhibiting the binding of an integrin to a cognate ligand in a subject comprising administering to said subject an effective amount of an antibody, or an antigen binding fragment thereof, which selectively binds to an integrin I-domain in the open conformation, thereby inhibiting the binding of an integrin to a cognate ligand in a subject.

63. The method of claim 62, wherein said antibody is an LFA-1 antibody, or an antigen binding fragment thereof.

64. The method of any one of claims 54, 59, or 62, wherein said antibody, or an antigen binding fragment thereof, binds to an activation specific epitope on the integrin I-domain.

65. A vaccine formulation for prophylactic or therapeutic treatment of an inflammatory disorder comprising an effective amount of a nucleic acid encoding a modified integrin I-domain polypeptide, or active fragment thereof.

66. The vaccine formulation of claim 65, further comprising an antigenic component.

67. The vaccine formulation of claim 65, further comprising a pharmaceutically acceptable carrier.

68. A method for treating an integrin-mediated disorder in a subject comprising administering to said subject a nucleic acid molecule encoding a modified integrin I-domain polypeptide, or active fragment thereof, inserted into a vector.

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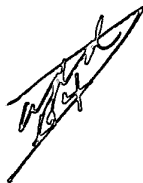
69. The method of claim 68, wherein said nucleic acid molecule is administered to a subject by intravenous injection.

70. The method of claim 68, wherein said nucleic acid molecule further comprises an antigenic component.

71. A non-human, transgenic animal comprising a nucleic acid molecule encoding a modified integrin I-domain polypeptide.

72. The transgenic animal of claim 71, wherein said animal is a mouse.

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